Claims

1. A method for identifying and isolating a gene of interest from a gene library, wherein the gene encodes a polypeptide carrying a signal sequence for secretion or partial secretion, the method comprising the steps of:

(a) providing a genomic DNA library or a cDNA library;

(b) inserting into said library a DNA fragment comprising a promoterless and secretion signal-less polynucleotide encoding a secretion reporter;

(c) introducing the library comprising the inserted DNA fragment into a host cell;

(d) screening for and selecting a host cell that secretes or partially secretes the active secretion reporter;

(e) identifying the gene of interest into which the secretion reporter was inserted in the selected host cell, by sequencing the DNA flanking the inserted DNA fragment; and

(f) isolating the complete gene of interest identified in step (e).

2. The method of claim 1, wherein the complete gene of interest in step (f) is isolated from the library of step (a).

3. The method of claim 1, wherein step (b) is performed *in vitro*.

4. The method of claim 1, wherein the cDNA or the cDNA library is normalized.

5. The method of claim 1, wherein the genomic DNA library or cDNA library is derived from a microorganism.

6. The method of claim 5, wherein the microorganism is a fungus.

7. The method of claim 6, wherein the fungus is a filamentous fungus or a yeast.

8. The method of claim 5, wherein the microorganism is a bacterium.

9. The method of claim 5, wherein the microorganism is an archaeon.

10. The method of claim 1, wherein the genomic DNA library or cDNA library is derived from a multicellular organism.

11. The method of claim 10, wherein the genomic DNA library or cDNA library is derived from a mammalian cell.

12. The method of claim 11, wherein the genomic DNA library or cDNA library is derived from a human cell.

13. The method of claim 1, wherein the DNA fragment comprises a transposon.

14. The method of claim 13, wherein the transposon is a MuA transposon.

15. The method of claim 1, wherein the DNA fragment comprises an origin of replication which is functional in the host cell.

16. The method of claim 15, wherein the origin of replication is functional in *Escherichia coli*.

17. The method of claim 16, wherein the origin of replication is a derivative of colE1, oriV, P15A, or colDF13.

18. The method of claim 17, wherein the origin of replication is colE1.

19. The method of claim 1, wherein the secretion reporter is a protein which, when secreted from the host cell, allows said cell to grow in the presence of a substance which otherwise inhibits growth of said cell.

20. The method of claim 19, wherein the secretion reporter is a β-lactamase or an invertase.

21. The method of claim 1, wherein the polynucleotide of the DNA-fragment of step (b) encodes a secretion reporter carrying an N-terminal peptide linker which comprises a specific target site for proteolytic cleavage.

22. The method of claim 1, wherein the host cell is bacterial.

23. The method of claim 22, wherein the bacterial host cell is a *Bacillus*, *Enterococcus*, *Escherichia*, *Lactococcus*, or *Streptomyces* cell.

24. The method of claim 23, wherein the bacterial host cell is a *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus brevis*, *Bacillus circulans*, *Bacillus clausii*, *Bacillus coagulans*, *Bacillus lautus*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus stearothermophilus*, *Bacillus subtilis*, *Bacillus thuringiensis*, *Enterococcus faecalis*, *Escherichia coli*, *Lactococcus lactis*, *Streptomyces coelicor*, or *Streptomyces griseus* cell.

25. The method of claim 1, wherein the host cell is fungal.

26. The method of claim 25, wherein the fungal host cell is an *Acremonium*, *Aspergillus*, *Aureobasidium*, *Candida*, *Cryptococcus*, *Filibasidium*, *Fusarium*, *Humicola*, *Kluyveromyces*, *Magnaporthe*, *Mucor*, *Myceliophthora*, *Neocallimastix*, *Neurospora*, *Paecilomyces*, *Penicillium*, *Pichia*, *Piromyces*, *Saccharomyces*, *Schizosaccharomyces*, *Schizophyllum*, *Talaromyces*, *Thermoascus*, *Thielavia*, *Tolypocladium*, *Trichoderma*, or *Yarrowia* cell.

27. The method of claim 26, wherein the fungal host cell is an *Aspergillus aculeatus*, *Aspergillus awamori*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus oryzae*, or *Saccharomyces cerevisiae* cell.

28. The method of claim 1, wherein the host cell is mammalian.

29. The method of claim 1, wherein the sequencing step of step (e) is performed using at least one primer directed to the DNA fragment , or using at least one primer directed to a vector in which the DNA library or cDNA library is cloned.

30. The method of claim 1, where isolating the complete gene of interest is done utilizing the DNA sequence information obtained in the sequencing step of step (e).

31. The method of claim 1, wherein the complete gene of interest encodes an enzyme that is secreted from the host cell.

32. The method of claim 1, wherein the complete gene of interest encodes a membrane-bound receptor.

33. The method of claim 32, wherein the complete gene of interest encodes a two-component signal (TCS) transduction receptor.

34. The method of claim 33, wherein the complete gene of interest encodes a cytokine receptor.

35. The method of claim 1, wherein the complete gene of interest encodes a secreted polypeptide cytokine.

36. The method of claim 1, wherein the complete gene of interest encodes a polypeptide which elicits an immunogenic response in humans.

37. The method of claim 1, wherein the complete gene of interest encodes a bacteriocin.

38. The method of claim 1, wherein the complete gene of interest encodes a plant pathogenic polypeptide.

39. The method of claim 1, further comprising constructing an expression system which comprises the complete gene of interest isolated in step (f)

40. A gene of interest, wherein said gene is isolated by the method of claim 1.

41. The gene of interest of claim 40, wherein the gene is isolated from a gene library.

42. An enzyme encoded by a gene of interest of claim 40.

43. An expression system comprising a gene of interest of claim 40.

44. A host cell comprising an expression system of claim 43.

45. A host cell comprising at least two chromosomally integrated copies of a gene of interest of claim 40.

46. A process for producing a polypeptide comprising cultivating a host cell of claim 44 under conditions suitable for expressing a gene of interest, wherein said host cell secretes a polypeptide encoded by said gene into the growth medium.

47. The process of claim 46, wherein the polypeptide is an enzyme.

48. The process of claim 46, further comprising purifying the polypeptide.